REMARKS

Entry of the foregoing, reexamination and reconsideration of the above-identified application is respectfully requested.

Claims 1 and 33 have been amended to specify that the microorganism employed in the claimed process is of the species *Mortierella alpina*. In addition, steps of collecting cultured cells and extracting the arachidonic acid or lipid containing same have been added. No new matter is added by these amendments. In addition, claims 3-5, 31 and 32 have been deleted without prejudice or disclaimer of the subject matter set forth therein. New claim 34 has been added directed to the specific embodiment of Example 2 of the specification. No new matter has been added. Moreover, such a claim is indicated in the Official Action to be allowable. Consideration of this claim is thus requested.

Claims 1-7 and 31-33 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not described in the specification. No basis or support is allegedly provided for the production of 7 g/l arachidonic acid under the conditions specified with any strain. The claims as amended specify that the microorganism is of the species *Mortierella alpina*. Example 2 of the specification is an example of the production of "about 7 g/l" arachidonic acid under the conditions specified using a strain of the species *Mortierella alpina*. The 7.1 g/L obtained would be "about 7 g/L" as recited in the claims. The specification further discloses the production of "at least about 7 g/L" at page 6, line 20 - page 7, line 2. Based upon Example 2, as well as additional descriptions in the specification such as at pages 6-7, one skilled in the art would recognize that applicants were in possession of a process as claimed, wherein a microorganism of the species

Mortierella alpina was used under the specified conditions and production of at least about 7 g/l arachidonic acid was obtained. No new matter is thus recited in the claims.

Withdrawal of this rejection is thus respectfully requested and believed to be in order.

Claims 1-7 and 31-33 have been rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. This rejection is respectfully traversed.

Claims 1 and 32 are said to be substantial duplicates. This aspect of the rejection is now moot in view of the deletion of claim 32.

Claim 31 is said to be vague and indefinite. This aspect of the rejection is now moot in view of the deletion of claim 31.

The claims were further said to be vague, indefinite and confusing as "it is unclear which strains of fungi are properly classifiable as members of the genus *Mortierella,* subgenus *Mortierella.*" This aspect of the rejection is rendered moot by the instant amendment, since the claims are now limited to the species *Mortierella alpina.* Moreover, it is respectfully believed that the members of the genus/subgenus would be sufficiently clear to a person skilled in the art. These classes of microorganism are well defined in Microbiology textbooks and are well recognized in the art.

Claims 1-7 and 31-33 are said to be incomplete in the absence of a recovery step. Independent claims 1 and 33 have been amended to recite recovery steps, i.e., collecting the cells and extracting the arachidonic acid or lipid containing same.

Withdrawal of this rejection is thus respectfully requested and believed to be in order.

Claims 31-32 were rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Kyle. This rejection is now moot in view of the deletion of these claims.

Claims 1, 3-7 and 31-33 have been rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Kyle. This rejection is respectfully traversed.

In the Official Action, it is stated that the results of Example 2 cannot be extrapolated to any strain of *Mortierella* having resistance to a carbon source of high concentration. Moreover, it is stated that there is nothing to show that the strain of Kyle does not have the carbon source resistance. Submitted herewith is an "Experiment Report." This Report shows that a second strain of *Mortierella alpina* in addition tot he SAM 2197 strain is (1) resistant to carbon, and (2) is capable of producing arachidonic acid at a rate of 7 g/L. This confirms applicants' teachings in the specification that the microorganism of the invention are capable of producing arachidonic acid at such rates.

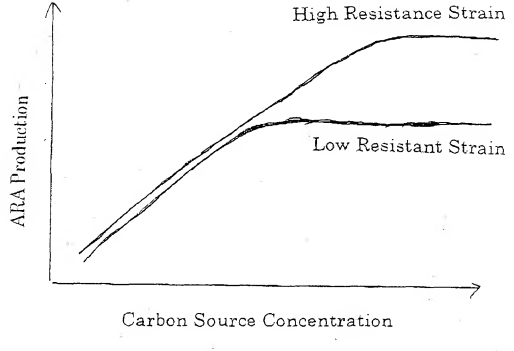
Moreover, this Report shows that the strain employed in Kyle, the ATCC42430 strain, does *not* produce arachidonic acid at the claimed rate. Instead, only 4.0 g/L arachidonic acid is produced by the *Mortierella alpina* strain of Kyle.

The results of the enclosed "Experiment Report" will be presented in the form of a Declaration. In the event that the signed Declaration is not received upon consideration of this application by the Examiner, a telephone call to the undersigned applicants' representative is respectfully requested.

It is noted that, while the strain CBS608.70 was known prior to the priority date of the instant application, its resistance to carbon source was not known. Thus, there would

have been no motivation to use the strain in a process as instantly claimed. Therefore, its use in a process as instantly claimed was not taught or even suggested in the prior art.

It is noted that, theoretically, the maximum amount of cultured cells increases as the total concentration of a carbon source in a culture medium increases. As a result, the total amount of product, i.e., arachidonic acid, increases as the total concentration in culture medium increases. However, in practice, a high concentration of carbon source inhibits the growth of the producer microorganism. As a result, the maximum level of product is limited. In the case where a microorganism having lower resistance to carbon source concentration is used, the growth of cells stops at a lower level of cell concentration, even if a culture medium contains a high level of carbon source concentration, due to inhibition by the carbon source. As a result, the maximum level of the product is low. However, when a microorganism having a higher resistance to carbon source concentration is used, the growth of cells stops at a higher level of cell concentration, if a medium contains a high level of carbon source concentration. As a result, the maximum level of the product obtained is high. Schematically, this is shown as follows:



Prior to the instant invention, the microorganisms used for producing arachidonic acid had a low resistance to a high glucose concentration. These microorganism show a low growth level of microorganism, resulting in a low production amount of the lipid . While a high concentration of carbon source is desirable as a nutrition source, increasing the carbon concentration produces harsh conditions for growing the microorganism due to the resultant osmotic pressure, and results in suppressing the growth thereof. *See,* page 4, lines 3-17 of the specification.

In order to overcome such problems, applicants discovered a method whereby arachidonic acid could be produced in large amount, i.e., at least about 7 g/L. Applicants discovered that microorganisms having resistence to high carbon concentrations could be employed and high amounts of arachidonic acid could be obtained, using a medium having a high initial glucose concentration. *See,* page 4, lines 28-36. As noted at page 6, lines 1-9 of the specification,

> [A]t 4% by weight of carbon source concentration, conventional microorganism belonging to the genus *Mortierella,* subgenus *Mortierella* (such as *Mortierella alpina* IFO 8568) and having been conventionally used for the production of arachidonic acid, ... lower their growth level, and at 8% by weight of carbon source concentration, the above-mentioned conventional microorganisms cannot grow.

Such a process whereby high amounts of arachidonic acid can be produced using a high concentration of carbon source in the medium thus was never disclosed or suggested, prior to applicants' invention.

In view of the above, withdrawal of the rejection of record under §103(a) is respectfully requested and believed to be in order.

In the event that there are any questions relating to this amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (508) 339-3684 so that prosecution of the application may be expedited.

Respectfully submitted,

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Date: December 16, 2002

Attachment to Reply and Amendment dated December 16, 2002

Marked-up Claims 1 and 33

1. (Four-Times Amended) A process for producing arachidonic acid or a lipid containing arachidonic acid comprising the steps of:

(1) culturing a microorganism, belonging to the [genus] species *Mortierella alpina* and having resistance to a carbon source of high concentration, in a medium having a carbon source concentration of at least 4% by weight at the start of culturing and the addition of at least an additional 6% by weight of carbon source during the culturing, thereby forming arachidonic acid or a lipid containing arachidonic acid;

(2) collecting the cultured cells; and

(3) extracting arachidonic acid or a lipid containing arachidonic acid from the collected cells;

wherein the microorganism produces arachidonic acid of at least about 7 g/L culture medium when cultured in a medium containing at least about 4% carbon source at the start of culturing and the addition of at least an additional 6% by weight of carbon source during the culturing, and at least about 2% nitrogen source at the start of culturing for 5 to 10 days with agitation and aeration.

33. (Amended) A process for producing arachidonic acid or a lipid containing arachidonic acid comprising the steps of:

(1) culturing a microorganism, belonging to the [genus] species *Mortierella alpina* and having resistance to a carbon source of high concentration, in a medium having

Attachment to Reply and Amendment dated December 16, 2002

Marked-up Claims 1 and 33

a carbon source concentration of at least 4% by weight at the start of culturing and the addition of at least an additional 6% by weight of carbon source during the culturing, thereby forming arachidonic acid or a lipid containing arachidonic acid;

(2) collecting the cultured cells; and

(3) extracting arachidonic acid or a lipid containing arachidonic acid from the collected cells;

wherein the microorganism produces arachidonic acid of at least about 7 g/L culture medium when cultured in a medium containing at least about 4% carbon source at the start of culturing and the addition of at least an additional 6% by weight of carbon source during the culturing, and at least about 2% nitrogen source at the start of culturing for about 5 to 10 days with agitation and an aeration rate of at least about 1 vvm.

EXPERIMENT REPORT

Microorganisms used for experiments

Three kinds of strains were used for this experiment. *M.alpina* SAM2197 and *M. alpina* CBS608.70 were chosen as strains having resistance to high concentration of carbon source, and *M. alpina* ATCC42430 as strain not having that.

Mortierella alpina SAM2197
Mortierella alpina CBS608.70
Mortierella alpina ATCC42430 (3 strains in total)

Materials and Methods

Five liter of the following medium was prepared in a 10-liter jar fermentor.

Glucose 4%, Yeast extract 2%, Soybean oil 0.2%, Adekanol 0.01%, pH 6.3

The medium was sterilized at 120℃ for 30 minutes. One hundred milliliters of a preculture solution of the strains was inoculated into the sterilized medium, and culturing with aeration and agitation was conducted for 9 days at 28℃ at an aeration rate of 1 vvm with stirring at 300 rpm.

Glucose of 2% (as concentration in culture broth) were added on the second, the third and the fourth day of culturing. After completion of the culture, the amount of arachidonic acid produced were determined using the same method as an example of Suzuki et al. (PCT patent, WO98/39468, see Example 1).

Results

M. alpina SAM2197 and *M. alpina* CBS 608.70 have resistance to a carbon source of high concentration, therefore the amounts of arachidonic acid produced were higher than 7 g/L. However, *M. alpina* ATCC42430 does not have resistance to a carbon source of high concentration, and the amount of arachidonic acid produced was lower than 7 g/L.

Table 1. Results

Strain	Amount of arachidonic acid produced
Mortierella alpina SAM2197	7.4 g/L
Mortierella alpina CBS608.70	7.2 g/L
Mortierella alpina ATCC42430	4.0 g/L

Date: November 12, 2002
Experimenter: Kengo Akimoto
Suntory Limited